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Heterospecific transformation in *Bacillus subtilis*

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Chapter VI

SUMMARY AND CONCLUDING REMARKS

Bacterial transformation is the genetic modification of bacteria following their exposure to exogenous DNA. Both the study of the absorption of DNA molecules by a recipient cell and the intracellular processing of entered DNA, resulting in the expression of its genetic information, is of interest in understanding the communication of the cell with its environment. Particularly, the integration of donor DNA into the recipient chromosome, which is a prerequisite for transforming activity of entered chromosomal DNA, provides an interesting tool for the study of general recombination *in vivo* at the molecular level.

Most of the knowledge concerning the mechanisms underlying bacterial transformation has emerged from the isolation and characterization of mutants impaired in the processing of transforming DNA and the study of the molecular fate of entered donor DNA. Transformation of *Bacillus subtilis* is initiated by the adsorption of double-stranded donor DNA from the surrounding medium by a DNA-binding protein, which is part of a membrane-located multimeric protein complex. The other component of this complex, a DNA-nuclease, is believed to be involved in the degradation of one of the strands of the donor DNA molecule and the simultaneous entry of the complementary strand. Entered single-stranded donor DNA fragments are integrated into the recipient chromosome, which is promoted by the Rec E gene product. Integration initially involves exposure of recipient base-sequences and nucleation at homologous regions, followed by heteroduplex extension and degradation of the displaced recipient strand. Maturation of this recombination intermediate is achieved by the formation of phosphodiester bonds between the extremities of the donor moiety and the recipient chromosome.

Undoubtedly, base sequence homology plays a decisive role in the integration process. This is reflected by an extremely low efficiency of hetero-specific transformation, in which the donor DNA is obtained from a different, though related species (Chapter II).

The investigations presented in this thesis, aimed at the elucidation of the intracellular processing of heterologous chromosomal DNA, emphasize the role of base-sequence inhomology in the interaction with the recipient chromosome (Chapter II + III). The observation that this interaction remains fixed at an initial stage, presented the opportunity to investigate the role

of the cellular membrane in the recombination process (Chapter IV) and the location of proteins known to be involved in DNA entry and in recombination (Chapter V).

Chapter II describes the molecular fate of *B. pumilus* DNA and *B. licheniformis* DNA entered into competent *B. subtilis*. The DNAs of these donor strains hybridized with *B. subtilis* DNA at a rate of 24% and 11%, respectively, and showed very little transforming activity. The experimental procedure employed to analyse the physical state of entered heterologous donor DNA allowed the detection of associations between donor and recipient DNA that resist heating at 70°C and exposure to pH 11.2. These associations are referred to as *stable* complexes. The formation of stable complexes was restricted to a minor fraction of the entered heterologous donor DNA, dependent on the degree of homology between the donor and recipient DNA. However, most of the donor DNA engaged in this stable association was not covalently linked to the recipient chromosome and was, similar to the majority of entered heterologous donor DNA, subject to gradual degradation. Apparently, in addition to inhibiting the formation of long regions of base pairing between donor and recipient DNA, lack of base sequence homology also prevents covalent linkage of the donor DNA to the recipient chromosome.

Surprisingly, the results presented in Chapter III show that, whereas few stable complexes were formed, the majority of entered heterologous donor DNA efficiently associated with the recipient chromosome in an *unstable* way. This association did not resist heating at 70°C and exposure to pH 11.2, unless previously artificially stabilized by treatment with 4,5',8-trimethylpsoralen that produces crosslinks in double-stranded DNA. Unstable donor-recipient DNA complex formation depended on recombination-proficiency of the recipient strain and strand-separation of the recipient chromosome and, in addition, appeared to be related to the degree of homology between donor and recipient DNA, at least in a qualitative way. Therefore, unstable donor-recipient DNA complexes were interpreted as the fixation of an early recombination event in which the donor DNA is poorly associated with the recipient chromosome by short regions of base pairing.

This view is supported by the observation that deproteinization did not dissociate the unstable complexes (Chapter IV). Based on the high sensitivity of the donor DNA moiety in the complex to nuclease S1 and the rapid dissociation of the complex observed under moderate denaturing conditions, it is argued that the donor and the recipient DNA were associated either by several short sequences of perfectly matched basepairs or by longer regions

of base pair mismatches. The heterologous the integration and recipient initial nucleic heteroduplex donor and recipient

In Chapter transformation stable homolog complexes were homologous donor tion, heterolog brane, presumably

To examine transforming DNA be involved in fragments carrying ments. By means detected that v brane-DNA comp teins, the Rec protein comple This result su into the recipient donor DNA thro

of base pairing of about 200 nucleotides containing approximately 25% of mismatches. The results summarized thus far, indicate that discrimination of heterologous DNA during bacterial transformation acts *post-synaptically*: the integration system efficiently promotes initial nucleation between donor and recipient DNA, without the need for extensive homology. However, this initial nucleation between the two moieties of DNA is not followed by heteroduplex extension and the formation of phosphodiester bonds between donor and recipient DNA.

In Chapter IV evidence is presented that recombination during transformation of *B. subtilis* occurs at the cellular membrane, because stable homologous as well as unstable heterologous donor-recipient DNA complexes were initially found to be membrane-bound. However, unlike homologous donor recipient complex which was slowly released during incubation, heterologous donor-recipient complex was retained at the cellular membrane, presumably due to its predominantly single-stranded character.

To examine the involvement of the cellular membrane in the processing of transforming DNA in more detail, the membrane location of proteins known to be involved in this processing was investigated. To this aim membrane fragments carrying chromosomal DNA were separated from free membrane fragments. By means of twodimensional-gel electrophoresis, several proteins were detected that were uniquely present or considerably enriched in the membrane-DNA complex and not in the free membrane fragments. Among these proteins, the Rec E gene product involved in recombination, and the multimeric protein complex involved in binding and entry of donor DNA were present. This result suggests that the membrane sites at which donor DNA integrates into the recipient chromosome are in the vicinity of the sites of entry of donor DNA through the membrane (Chapter V).